AAL-TDR-62-63



# STUDIES ON THE POTENTIATION OF ENDOTOXIN IN MICE BY EXPOSURE TO COLD

TECHNICAL DOCUMENTARY REPORT AAL-TDR-62-63

May 1963



ARCTIC AEROMEDICAL LABORATORY

AEROSPACE MEDICAL DIVISION
AIR FORCE SYSTEMS COMMAND
FORT WAINWRIGHT, ALASKA

Project 8241-01

(Prepared under Contract AF 41 (657)-340 by
J.J. Previte and L.J. Berry
Bryn Mawr College, Bryn Mawr, Pa.)

## NOTICES

When US Government drawings, specifications, or other data are used for any purpose other than a definitely related government procurement operation, the government thereby incurs no responsibility nor any obligation whatsoever; and the fact that the government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data is not to be regarded by implication or otherwise, as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

US Government agencies and other qualified ASTIA users may obtain copies of this report from the Armed Services Technical Information Agency, Documents Service Center, Arlington 12, Virginia.

This report has been released to the Office of Technical Services, U. S. Department of Commerce, Washington 25, D. C., for sale to the general public.

Arctic Aeromedical Laboratory, United States Air Force (AFSC), APO 731, Seattle, Wash.
Rpt. AAL-TDR-62-63. STUDIES ON THE POTENTIATION OF ENDOTOXIN IN MICE BY EXPOSURE TO COLD. May 1963, 17 p. incl. tables, 41 refs.
Unclassified Report

Mice exposed to 5° C show a drop in body temperature at a time when they are more sensitive than normal mice to bacterial lipopolysaccharide (LPS). Injection of 5 mg cortisone acetate protects against the lethal effects while 8 units but not 2 units of ACTH enhance lethality. These data suggest that adrenocortical exhaustion due to cold is in part responsible for the altered sensitivity to LPS. In support of this interpretation is the increase in urine volume and urinary nitrogen excreted by mice at 5° C compared with those at 25° C. Endotoxin decreases both. The decrease is counteracted by cortisone administration but not by ACTH

1. Toxins and Antitoxins
2. Mice
3. Exposure
4. Body Temperature
5. Stress (Physiology)
6. Corticosteroid Agents
7. Adrenocorticotropic Hormone
8. Urine
9. Nitrogen Compounds
I. Project 8241-01
II. Contract AF41(657)-340
III. Bryn Mawr College, Bryn Mawr, Pa.
IV. Previte, J. J. and L. J. Berry
V. Available from OTS
VI. In ASTIA collection

Arctic Aeromedical Laboratory, United States Air Force (AFSC), APO 731, Seattle, Wash.
Rpt. AAL-TDR-62-63. STUDIES ON THE POTENTIATION OF ENDOTOXIN IN MICE BY EXPOSURE TO COLD. May 1963, 17 p. incl. tables, 41 refs.
Unclassified Report

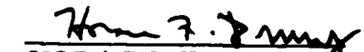
Mice exposed to 5° C show a drop in body temperature at a time when they are more sensitive than normal mice to bacterial lipopolysaccharide (LPS). Injection of 5 mg cortisone acetate protects against the lethal effects while 8 units but not 2 units of ACTH enhance lethality. These data suggest that adrenocortical exhaustion due to cold is in part responsible for the altered sensitivity to LPS. In support of this interpretation is the increase in urine volume and urinary nitrogen excreted by mice at 5° C compared with those at 25° C. Endotoxin decreases both. The decrease is counteracted by cortisone administration but not by ACTH

1. Toxins and Antitoxins
2. Mice
3. Exposure
4. Body Temperature
5. Stress (Physiology)
6. Corticosteroid Agents
7. Adrenocorticotropic Hormone
8. Urine
9. Nitrogen Compounds
I. Project 8241-01
II. Contract AF41(657)-340
III. Bryn Mawr College, Bryn Mawr, Pa.
IV. Previte, J. J. and L. J. Berry
V. Available from OTS
VI. In ASTIA collection

## ABSTRACT

Mice exposed to 5° C show a drop in body temperature at a time when they are more sensitive than normal mice to bacterial lipopolysaccharide (LPS). Injection of 5 mg cortisone acetate protects against the lethal effects while 8 units but not 2 units of ACTH enhance lethality. These data suggest that adrenocortical exhaustion due to cold is in part responsible for the altered sensitivity to LPS. In support of this interpretation is the increase in urine volume and urinary nitrogen excreted by mice at 5° C compared with those at 25° C. Endotoxin decreases both. The decrease is counteracted by cortisone administration but not by ACTH.


## PUBLICATION REVIEW



HORACE F. DRURY
Director of Research

# STUDIES ON THE POTENTIATION OF ENDOTOXIN IN MICE BY EXPOSURE TO COLD

## SECTION 1. INTRODUCTION

Exposure to low ambient temperature is known to predispose experimental animals, and possibly man, to infectious diseases under certain conditions (Girone, 1962). In a recent report, Previte and Berry (1962) found cold exposed mice to be more susceptible to infectious challenge with Salmonella typhimurium or Staphylococcus aureus when strains of low virulence but not those of high virulence were employed. This finding has been confirmed by Miraglia and Berry (1962, 1963) who, in addition, implicated the cellular defense as a factor in the altered response to the pathogen. Not only does cold exposure alter host-parasite interaction but it also renders mice more susceptible to the lethal effects of bacterial endotoxins derived from Gram-negative organisms (Previte and Berry, 1962). Since infections with Gram-negative organisms are believed to result in symptoms associated with endotoxins released from the bacterial cell (Berry and Smythe, 1960), it is important to pursue in greater detail the relationship between cold exposure and altered susceptibility to endotoxins.

This raises the question of a common site of action for cold and bacterial endotoxins. Cold, for example, is known to produce physiological alterations (Culver, 1959; South, 1960; Fuhrman and Fuhrman, 1961; Hardy, 1961; Carlson, 1962) which might be fundamental in determining the response of an animal not only to toxins but to infectious challenge as well. The latter becomes reasonable in light of the profound influence that changes in host metabolism are known to have on host-parasite interactions (Smith and Dubos, 1956; Bauer et al, 1956; Berry and Mitchell, 1953a, 1953b, 1954; Berry et al, 1954a, 1954b; Berry, 1956; Garber, 1960). Because of the significance of specific changes in adrenal cortical activity in response to cold stress (Heroux and Hart, 1954a, 1954b) and to endotoxin poisoning (Duffy and Morgan, 1951), the behavior of cold exposed endotoxin poisoned mice treated with either cortisone acetate or adrenocorticotrophic hormone (ACTH) is analyzed in this report. The results indicate that "functional adrenalectomy" due to cold exposure may be paramount in the sensitization of cold exposed mice to bacterial lipopolysaccharide.

## SECTION 2. SUMMARY

The period during which sensitization to lipopolysaccharide occurs in mice following 5° C exposure (6 to 12 hours after cold stress) is paralleled by the time at which a drop in body temperature occurs following cold exposure and/or endotoxin poisoning.

An initial depletion of corticoid reserves may occur on exposure to cold prior to the time when the hormones are needed for protection against endotoxin. Thus, "functional adrenalectomy" due to temperature stress may be responsible for sensitization of cold exposed mice to endotoxin (lipopolysaccharide) just as surgical adrenalectomy has been reported to do so in rats. Release of adrenal corticoids within the six-hour experimental period was inferred by a rise in volume of urine excreted and an increase in levels of urinary nitrogen following cold exposure. Endotoxin caused urinary nitrogen to decrease below control levels at 5° or 25° C. Exogenously administered cortisone acetate protected mice against endotoxin poisoning and allowed them to maintain close to normal levels of urinary nitrogen when poisoned with lipopolysaccharide. Eight units of exogenously administered ACTH enhanced the lethal effects of lipopolysaccharide, while two units had little effect in terms of lethality of a given dose of endotoxin. The lack of a significant effect of two units of ACTH may have been due to insufficient absorption of the hormone within the time of the experiment. Carcass non-protein nitrogen did not change significantly within six hours under any of the experimental conditions described above.

While this report stresses the involvement of adrenal cortical factors in protection or sensitization to endotoxin following cold exposure, it is recognized that more data must be gathered concerning other facets of endocrine involvement, metabolism and host defenses before definitive statements can be made concerning this problem.

## SECTION 3. METHODS

Exposure to Environmental Temperature. A walk-in refrigerator maintained at 5° ± 1° C was used as a low temperature environment and an air-conditioned laboratory held at 25° ± 2° C was used as the control. During the period of actual experimentation the mice were housed individually

without bedding in compartments fabricated of 1/8 inch plexiglass sheets. A total of 10 mice were contained in an area of 8 X 10 1/2 inches. The housing and exposure conditions are described in detail elsewhere (Previte and Berry, 1962). Each mouse was provided with a small food trough capable of holding food for a two-day period. Water and food (Dietrich and Gambrill's pathogen-free mouse biscuits) were available for each animal at all times.

Bacterial Endotoxin. Lipopolysaccharide derived from Serratia marcescens was used as a source of endotoxin (Difco Laboratories, Detroit). Mice were injected via the intraperitoneal route with the desired dose dissolved in 0.5 ml of nonpyrogenic saline (Baxter Laboratories). The $LD_{50}$ of endotoxin for mice housed at room temperature is approximately 10 times that for animals maintained at 5° C (Previte and Berry, 1962). In the majority of experiments to be described, doses approximating the $LD_{50}$ were used.

Rectal Temperatures. Rectal temperatures were determined by inserting a thermister probe approximately 21 mm into the rectum of mice for 20 seconds and reading the temperature with a telethermometer (Yellow Springs Instrument Company, Yellow Springs, Ohio) to which the probe was connected. The instrument was calibrated against a sensitive mercury thermometer.

Hormones. Five mg of cortisone were administered subcutaneously as a suspension of cortisone acetate (Nutritional Biochemicals, Cleveland, Ohio) in 0.5 ml of nonpyrogenic saline. The suspension, stabilized with a drop of Tween 80, was prepared in a glass homogenizer with teflon pestle and used immediately thereafter.

ACTH was injected subcutaneously as a gelatin suspension (Armour's Acthar Gel) containing two units per 0.05 ml.

Urinary Nitrogen Determination. The method of collecting urine is a modification of that described by Berry and Smythe (1959). Stainless steel wire cloth, 16 mesh, was used to construct a base large enough to support the plexiglass compartments for 10 mice. The units were placed over a polyethylene funnel measuring 280 mm in diameter. The bladder of each animal was voided by applying digital pressure to the lower abdomen prior to placing them in the metabolism cages. Urine from six mice was collected in a 15 ml graduated centrifuge tube containing 3 ml of toluene, into which the stem of a glass funnel which measured 55 mm in diameter was inserted. The glass funnel served to connect the large polyethylene funnel

with the centrifuge tube. The volume of urine was recorded and nitrogen was determined by microKjeldahl analysis. Each value represents, therefore, the average urinary nitrogen excreted by six animals. A collecting period of six hours was used in these studies, during which both food and water were withheld, because deaths at 5° C commence about seven hours after the injection of an $LD_{50}$ dose of endotoxin. At least five groups of six mice were used for the values presented in the results, expressed as mg nitrogen per mouse per six-hour period.

Carcass Nonprotein Nitrogen in the Mouse. About six hours after an experimental treatment, the skinned, decapitated carcass with digestive tract, paws and tail removed was weighed and homogenized in a Waring blendor for two minutes in 10% trichloroacetic acid (TCA). The volume of acid in cubic centimeters added to the carcass was determined by subtracting the carcass weight in grams from 100. For example, an 11-gram carcass was homogenized with 89 ml of 10% TCA. An aliquot of the homogenate was centrifuged and 2 ml of the clear supernatant was assayed for nitrogen by the microKjeldahl procedure. The nonprotein nitrogen contained in the carcass was then calculated.

Statistical Methods. The significance of differences in survival due to experimental treatments was determined by the chi-square test using Yates' corrected formula (cf. Croxton, 1959) and that for nitrogen determinations was by the rank order test (White, 1952).

Mice. CF-1 female mice (Carworth Farms, New City, N. Y.) were used exclusively. Weekly shipments of animals weighing 16 to 18 grams were received. Groups of 10 animals were housed in small cages, bedded in pine shavings and kept in an animal room maintained at 25° ± 2° C until ready for study. They were fed Dietrich and Gambrill's pathogen-free mouse food ad libitum and water was available at all times. Mice were used when they weighed 23 ± 2 grams.

## SECTION 4. RESULTS

### Rectal Temperatures

Endotoxin is known to elicit hypothermia in mice (Halberg and Spink, 1956; Berry et al, 1959). The question remains as to whether the onset and duration of hypothermia coincide with the 6 to 12 hour period during which

mice exposed to cold are most sensitive to endotoxin (Previte and Berry, 1962). Rectal temperatures were measured, therefore, at different intervals in the four experimental groups shown in Table I. Animals exposed to 5° C for six hours had a body temperature of 36.1° versus that of 37.5° C in mice maintained at 25° ($P<0.001$). After 12 hours, body temperatures were 36.3° at 5° C and 37.9° at 25° C ($P<0.001$). After 24 hours in the cold, however, the mice held at 5° C showed no significant difference in rectal temperature from those held at 25° C. On the basis of these findings, exposure to 5° C causes an initial mild hypothermia which disappears within 24 hours.

TABLE I

Rectal temperatures of mice at different times following exposure to 25° C or to 5° C with and without an $LD_{50}$ dose of bacterial lipopolysaccharide. Each value is the mean ± the standard deviation for the number of separate determinations shown in parentheses.

| Time Post-Injection (hours) | Body Temperature of Mice Subjected to | | | |
|---|---|---|---|---|
| | 25° C | 25° C + 408 μg LPS | 5° C | 5° C + 50 μg LPS |
| 0 (9:15 A.M.) | 37.4 ± 0.5 (10) | | | |
| 6 | 37.5 ± 0.5 (10) | 36.4 ± 1.3 (10) | 36.1 ± 0.7 (10) | 29.6 ± 4.1* (20) |
| 12 | 37.9 ± 0.7 (10) | 34.8 ± 2.4 (10) | 36.3 ± 0.7 (10) | 29.7 ± 5.9* (9) |
| 24 | 36.6 ± 1.0 (10) | | 36.2 ± 0.6 (10) | |

* Rectal temperatures of moribund mice were often below the minimum of the telethermometer (20° C). They were listed as 20° in computing the means.

In mice maintained at 25° C the body temperature of controls (37.5° C) was significantly higher ($P<0.05$) than that of mice given an $LD_{50}$ dose of lipopolysaccharide six hours previously (36.4° C). After 12 hours the difference was even more marked between the two groups (37.9° vs 34.8° C, respectively) ($P<0.001$). The rectal temperatures of control mice exposed to 5° C and of mice given an $LD_{50}$ dose of lipopolysaccharide at the time of exposure to 5° C differ markedly at six hours (36.1° vs 29.6° C) and at 12 hours (36.3° vs 29.7° C) ($P<0.001$ in both cases). Hypothermia is clearly evident, therefore, six and 12 hours after the administration of lipopolysaccharide in animals maintained at 25° C and it is severe in animals housed at 5° C.

## Hormone Administration and Survivorship

Adrenalectomized rats are killed at about one-thousandth the dose of endotoxin required to kill normal animals (Brooke et al, 1959). Conversely, the administration of exogenous corticoids is known to protect animals against endotoxin, described as early as 1951 by Duffy and Morgan and confirmed since by Berry et al (1959) and others. The extent to which the same protection was evident in animals exposed to cold remained to be determined.

Five mg of cortisone acetate was administered subcutaneously immediately before intraperitoneal injection of 50 μg of lipopolysaccharide. Animals maintained at 5° C and treated with hormone showed slightly but not significantly greater survivorship ($P<0.05$) than animals receiving no hormone (89% vs 63%, Table II). With a larger quantity of endotoxin (70 μg), the protection afforded by cortisone was clearly demonstrated. All of 40 untreated mice died while 24 of 30 mice given 5 mg of cortisone acetate survived ($P<0.001$). Cortisone alone was without lethal effect.

Exogenous ACTH sensitizes mice to the lethal effects of endotoxin. This is, presumably, because the adrenal glycocorticoids are released too promptly to fulfill the protective role they play in endotoxin poisoning alone (Berry and Smythe, 1959, 1961b). The experiments described below were carried out to determine the extent to which cold exposed animals are further sensitized by ACTH to lipopolysaccharide.

Two units or eight units of ACTH were administered subcutaneously immediately before 50 μg of lipopolysaccharide. All mice were then exposed to 5° C and the results are given in Table III. Survival in controls (63%) was slightly but not significantly higher than that of animals receiving two units of ACTH (50%) but was significantly greater than that of animals receiving eight units of the hormone (10% survival) ($P<0.001$). Eight units of ACTH alone killed none of 14 mice.

TABLE II

Survival of mice at 5° C following the intraperitoneal injection of bacterial lipopolysaccharide with and without the subcutaneous administration of 5 mg cortisone acetate at the same time.

| Days Post-Injection | Number of Animals Surviving at Times Shown Following the Injection of | | | | |
|---|---|---|---|---|---|
| | 50 μg LPS | Cortisone + 50 μg LPS | 70 μg LPS | Cortisone + 70 μg LPS | Cortisone |
| 0 | 30 | 26 | 40 | 30 | 10 |
| 1 | 19 | 23 | 0 | 24 | 10 |
| 2 | 19 | 23 | 0 | 24 | 10 |
| Per Cent Survival | 63% | 89% | 0% | 80% | 100% |

TABLE III

Survival of mice at 5° C following the intraperitoneal injection of bacterial lipopolysaccharide with and without the subcutaneous administration of either 2 units or 8 units of ACTH at the same time.

| Days Post-Injection | Number of Animals Surviving at Times Shown Following the Injection of | | | |
|---|---|---|---|---|
| | 50 μg LPS | 50 μg LPS + 2 units ACTH | 50 μg LPS + 8 units ACTH | 8 units ACTH |
| 0 | 30 | 30 | 30 | 14 |
| 1 | 19 | 15 | 3 | 14 |
| 2 | 19 | 15 | 3 | 14 |
| Per Cent Survival | 63% | 50% | 10% | 100% |

## Urine Volume Excreted

Diuresis in experimental animals accompanies exposure to cold. Adrenal cortical secretions are believed to increase water elimination (Itoh et al, 1959). Therefore, urine volume was measured in our experiment as an assessment of adrenal cortical involvement. The results are presented in Table IV.

TABLE IV

Volume of urine excreted by mice maintained at either 25° C or 5° C and treated with lipopolysaccharide (LPS), 5 mg cortisone acetate, and 2 units of ACTH singly or in combination. Each value is the mean ± the standard deviation for the number of separate determinations (each on the pooled urine of six mice) shown in parentheses. The LPS was injected intraperitoneally and the hormones subcutaneously at the same time.

| Experimental Treatment | Urine Volume (ml/mouse/hr) excreted by mice housed at 25° C | 5° C |
|---|---|---|
| None (Controls) | 0.3 ± 0.04 (6) | 0.6 ± 0.11 (8) |
| LPS (500 µg at 25° C and 50 µg at 5° C) | 0.2 ± 0.04 (6) | 0.4 ± 0.08 (6) |
| Cortisone, 5 mg | 0.9 ± 0.13 (6) | 0.6 ± 0.22 (6) |
| LPS (as above) + cortisone, 5 mg | 0.7 ± 0.21 (5) | 0.8 ± 0.20 (6) |
| ACTH, 2 units | 0.4 ± 0.09 (11) | 0.5 ± 0.17 (7) |
| LPS (as above) + ACTH, 2 units | 0.07 ± 0.04 (7) | 0.2 ± 0.07 (6) |

The volume of urine excreted by mice at 5° C is twice that for animals at 25° C (line 1, columns 2 and 3) ($P = 0.001$). Urine excretion by mice poisoned with approximately an $LD_{50}$ dose of lipopolysaccharide is about half that of the corresponding controls ($P = 0.001$). In each case the percentage decrease in urine volume in mice given the lipopolysaccharide compared with controls is about the same, though at 5° C the dose of poison is only one-tenth that at 25° C.

According to the findings of Itoh et al (1959), cortisone administration to mice should increase urine volume. This was found to occur when 5 mg of cortisone acetate was injected subcutaneously in animals housed at 25° C but not in those kept at 5° C. Values at 25° C were nearly triple those of controls (line 1 vs line 3, column 2) ($P<0.01$) while at 5° C the exogenous hormone had no statistically significant effect (line 1 vs line 3, column 3) ($P<0.05$). The volume of urine produced under the influence of endogenous corticoid, the output of which was apparently elevated in response to cold stress, could not be augmented under the conditions of the experiment by cortisone acetate.

When cortisone was administered concurrently with lipopolysaccharide, urine volume increased to about two and one-half times the control levels at 25° C (line 1 vs line 4, column 2) ($P<0.01$) but increased only slightly at 5° C (line 1 vs line 4, column 3) ($P = 0.05$).

Two units of ACTH administered subcutaneously resulted in no appreciable change ($P<0.05$) in volume of urine secreted by mice at 25° C or at 5° C (line 1 vs line 5, columns 2 and 3). The same hormone given concurrently with lipopolysaccharide resulted in a volume of urine that was less than one-fourth that produced by control mice at 25° C (line 1 vs line 6, column 2) ($P<0.01$) and was about one-third that for controls at 5° C (line 1 vs line 6, column 3) ($P<0.001$).

From these data one may conclude that urine volumes are (a) elevated by cold exposure, (b) depressed by lipopolysaccharide, (c) increased by cortisone and (d) practically unaffected by ACTH. A combination of cortisone and lipopolysaccharide increased urine volume significantly above the control level in mice at 25° C but less dramatically, though still significantly, in mice at 5° C. On the other hand, within the period of six hours ACTH not only failed to elevate the abnormally low volume of urine excreted by endotoxin poisoned mice maintained at either temperature but produced even lower values.

## Carcass Nonprotein Nitrogen

It has been known for some time that adrenal cortical hormones promote negative nitrogen balance (Long et al, 1940). This is manifested as an elevation in urinary nitrogen excretion in mice given an injection of cortisone or ACTH. Endotoxin, which fails to elicit such an elevation, also fails to prevent it when cortisone is injected. ACTH also causes an increase in urinary nitrogen excretion, but with this hormone endotoxin prevents completely or lowers in proportion to dose the elevation in nitrogen excreted (Berry and Smythe, 1961a). Under these conditions, an increase in carcass nonprotein nitrogen (NPN) is commonly observed (Berry and Smythe, 1961b). In light of these findings, changes in nitrogen metabolism were determined in various experimental groups as a means of assessing further the adrenal cortical involvement in the response of mice to lipopolysaccharide at low environmental temperature. The amount of carcass NPN was determined about six hours after experimental treatment and under all conditions the quantity observed was the same. Apparently, therefore, alterations in carcass NPN found 17 hours after procedures of the same type (Berry and Smythe, 1961b) are not evident within six hours. The treatments investigated are the same as those reported in the section below.

## Urinary Nitrogen Excretion

Urinary nitrogen excretion was determined in animals submitted to the experimental conditions presented in Table V. Each value listed represents mg nitrogen excreted per mouse per six hours.

Cold exposed normal mice excrete significantly more urinary nitrogen ($P<0.001$) than mice at room temperature (14.8 vs 11.1 mg, line 1). Urinary nitrogen excreted by controls at 25° C is significantly greater ($P<0.01$) than that for mice injected with 500 μg of lipopolysaccharide (11.1 vs 4.4 mg, lines 1 and 2, column 2). At 5° C nitrogen excretion dropped from 14.8 mg in controls to 7.9 mg in lipopolysaccharide poisoned mice (lines 1 and 2, column 3) ($P<0.001$).

No significant difference ($P<0.05$) in nitrogen excretion between controls and animals given both lipopolysaccharide and cortisone occurred in mice housed at 25° C (lines 1 and 4, column 2), while a slight decrease ($P = 0.05$) was found at 5° C (lines 1 and 4, column 3). Cortisone alone caused a slight but significant increase ($P = 0.05$) in urinary nitrogen excreted by animals exposed to 25° C but not in those housed at 5° C ($P<0.05$) (lines 1 and 3, columns 2 and 3). Thus, cortisone increased urinary

nitrogen above control levels in 25° C exposed animals but did not do so at 5° C. When administered to endotoxin poisoned mice housed at either temperature, cortisone reversed their characteristic abnormally low urinary nitrogen excretion.

TABLE V

Urinary nitrogen excreted by mice maintained at either 25° C or 5° C and treated with lipopolysaccharide (LPS), 5 mg cortisone acetate and 2 units of ACTH singly or in combination. Each value is the mean ± the standard deviation for the number of separate determinations (each on the pooled urine of six mice) shown in parentheses. The LPS was injected intraperitoneally and the hormones subcutaneously at the same time.

| Experimental Treatment | Urinary nitrogen (mg/mouse/6 hrs) excreted by mice housed at 25° C | 5° C |
|---|---|---|
| None (controls) | 11.1 ± 1.6 (6) | 14.8 ± 2.1 (8) |
| LPS (500 μg at 25° C and 50 μg at 5° C) | 4.4 ± 1.0 (6) | 7.9 ± 1.1 (6) |
| Cortisone, 5 mg | 13.1 ± 1.4 (6) | 12.2 ± 3.0 (6) |
| LPS (as above) + cortisone, 5 mg | 9.8 ± 1.6 (5) | 11.7 ± 1.9 (6) |
| ACTH, 2 units | 10.9 ± 1.6 (11) | 10.4 ± 1.8 (7) |
| LPS (as above) + ACTH, 2 units | 2.0 ± 1.0 (7) | 8.9 ± 3.2 (6) |

Within six hours of injection of two units of ACTH, no significant change from that of controls was observed in urinary nitrogen excretion in animals kept at 25° C (lines 1 and 5, column 2). Urine collected for

17 hours at 25° C following injection of ACTH contains about twice the nitrogen that normal mice excrete (Berry and Smythe, 1959, 1961a). A significant drop ($P<0.001$) was evident in ACTH treated animals housed at 5° C (lines 1 and 5, column 3). The basis for the latter effect is not known. When ACTH and an $LD_{50}$ dose of lipopolysaccharide were administered, the amount of urinary nitrogen excreted was significantly lower than that observed in controls kept at both 5° C and 25° C ($P<0.01$) (lines 6 and 1, columns 2 and 3).

Therefore, within six hours, two units of ACTH seem to have little effect at 25° C, while diminishing the urinary nitrogen excreted at 5° C. When this hormone is administered along with lipopolysaccharide, the decrease in urinary nitrogen excreted can be largely accounted for by the effects of the lipopolysaccharide alone (compare lines 1, 2 and 6, columns 2 and 3).

## SECTION 5. DISCUSSION

An explanation of why mice exposed to cold become more susceptible to the lethal effects of bacterial endotoxin is probably to be sought in changes induced in adrenal cortical function. This is substantiated, in part, by the fact that exogenously administered cortical hormones afford protection against endotoxin alone (Brooke et al, 1959), against cold stress alone (Heroux and Hart, 1954a), or against both factors combined (Table II). Moreover, the greater resistance of cold acclimatized mice to endotoxin compared with nonacclimatized animals (Previte and Berry, 1962) may be related to the fact that endogenous adrenal cortical activity levels off after an initial rise following cold exposure (Heroux and Hart, 1954b). A greater capacity of the adrenals to release sufficient protective corticoids at the proper time may be the crucial characteristic of the acclimatized animals.

In rats subjected to 5° C, the maximal activity of the adrenal cortex as determined by $P^{32}$ uptake occurs two hours after exposure (Rossiter and Nicholls, 1957). It seems possible, therefore, that initial depletion of corticoid reserves occurs in the cold prior to the time the hormones are needed for protection against endotoxin. This may be a major factor in rendering cold exposed animals more susceptible to endotoxin than mice maintained at room temperature. "Functional adrenalectomy" due to temperature stress may be responsible, therefore, for sensitization of cold exposed mice to endotoxin, just as surgical adrenalectomy renders

rats hyperreactive to endotoxin (Brooke et al, 1959). Commensurate with this concept is the obvious enhancement of lethality to endotoxin in cold exposed mice injected with eight units but not with two units of ACTH (Table III). Apparently adrenocortical stores become sufficiently depleted by the large dose of ACTH to render further cortical secretion impossible at a time when it is critically needed.

Sensitivity to endotoxin, at least in part, is believed to be determined at a metabolic level by the inability of the poisoned animal to maintain adequate levels of carbohydrate (Kun, 1948; Kun and Miller, 1948; Berry and Smythe, 1959). Not only is this evidenced by the near exhaustion of muscle and liver glycogen but also by the lack of equivalence between protein catabolized and carbohydrate anabolized in response to cortisone injection in endotoxin poisoned mice. Control mice show, essentially, an increase in total body carbohydrate equal to the total protein loss (as calculated by the elevation in urinary nitrogen excretion) 17 hours after cortisone injection. However, endotoxin poisoned mice under otherwise identical conditions undergo identical protein catabolism but have far less carbohydrate (Berry and Smythe, 1961a; Berry et al, 1962).

Well might one ask the relevance of these findings to cold exposure. Hale and Mefferd (1961) in their study of rats fasted and housed at $2^{\circ}$ C for 24 hours found significant weight loss and high levels of excretion of several different amino acids. If this should also occur in cold exposed endotoxin poisoned mice, some of the energy normally available from catabolized protein might be lost because of elevated amino acid excretion. These pathways coupled with the greater energy requirements in animals maintained at low temperatures and the increased rate of carbohydrate catabolism (Barnett et al, 1960; Depocas and Masironi, 1960) would make carbohydrate shortage more critical than at room temperature. It is also reasonable to postulate an impairment in glyconeogenesis, particularly if reduced pyridine nucleotides or high energy phosphate are deficient, as recent unpublished findings suggest (Berry, unpublished).

To fully assess the sensitization of mice to lipopolysaccharide by cold exposure requires additional data. Further studies regarding adrenal cortical and adrenal medullary involvement are necessary, as is knowledge of the participation of other endocrines. This becomes the more obvious when one considers the significance of adrenal-thyroid interaction in the maintenance of homeostasis in cold exposed animals (Ingle, 1958; Fregly, 1960). The role that carbohydrate and fat metabolism play in these phenomena may also be critical.

## REFERENCES


1. Barnett, S. A., E. M. Coleman and B. M. Manly. Mortality, growth and liver glycogen in young mice exposed to cold. Quart. J. Exp. Physiol. 45:40-49, 1960.

2. Bauer, H., J. F. Flanagan and W. H. Sheldon. The effects of metabolic alterations on experimental Rhizopus oryzae (Mucormycosis) infection. Yale J. of Bio. and Med. 29:23-32, 1956.

3. Berry, L. J. Effect of components of the tricarboxylic acid cycle on bacterial infections. Ann. N. Y. Acad. Sci. 66:370-381, 1956.

4. Berry, L. J., K. H. Ehlers and R. B. Mitchell. The relation of the tricarboxylic acid cycle to bacterial infection. J. Infect. Dis. 94:152-158, 1954a.

5. Berry, L. J., P. Merritt and R. B. Mitchell. The relation of the tricarboxylic acid cycle to bacterial infection. J. Infect. Dis. 94:144-151, 1954b.

6. Berry, L. J. and R. B. Mitchell. The relation of the tricarboxylic acid cycle to bacterial infection. I. The effect of Salmonella typhimurium infections in mice. J. Infect. Dis. 93:75-82, 1953a.

7. Berry, L. J. and R. B. Mitchell. The relation of the tricarboxylic acid cycle to bacterial infection. II. The effect of fluoroacetate arsenite, citrate and succinate on Salmonella typhimurium infections in mice. J. Infect. Dis. 93:83-92, 1953b.

8. Berry, L. J. and R. B. Mitchell. Effect of malonate on bacteremia developed in mice artificially infected with Salmonella typhimurium. Proc. Soc. Exper. Biol. and Med. 85:209-211, 1954.

9. Berry, L. J. and D. S. Smythe. Effects of bacterial endotoxin on metabolism. II. Protein-carbohydrate balance following cortisone inhibition of intestinal absorption and adrenal response to ACTH. J. Exp. Med. 110:407-418, 1959.

10. Berry, L. J. and D. S. Smythe. Some metabolic aspects of host-parasite interactions in the mouse typhoid model. Ann. N. Y. Acad. Sci. 88:1278-1286, 1960.

11. Berry, L.J. and D.S. Smythe. Effects of bacterial endotoxins on metabolism. III. Nitrogen excretion after ACTH as an assay for endotoxin. J. Exp. Med. 113:83-94, 1961a.

12. Berry, L.J. and D.S. Smythe. Effects of bacterial endotoxins on metabolism. IV. Renal function and adrenocortical activity as factors in the nitrogen excretion assay for endotoxin. J. Exp. Med. 114:761-778, 1961b.

13. Berry, L.J., D.S. Smythe and S. McC. Kolbye. Effects of bacterial endotoxins on metabolism. V. The hyperreactivity of mice infected with Mycobacterium tuberculosis, strain BCG. J. Exp. Med. 116:897-911, 1962.

14. Berry, L.J., D.S. Smythe and L.G. Young. Effects of bacterial endotoxins on metabolism. I. Carbohydrate depletion and the protective role of cortisone. J. Exp. Med. 110:389-405, 1959.

15. Brooke, M., E.H. Kass and O. Hechter. Protective effect of steroids against bacterial endotoxin. Fed. Proc. 18:560, 1959.

16. Carlson, L.D. Temperature. Ann. Rev. Physiol. 24:85-108, 1962.

17. Croxton, F.E. Elementary Statistics with Applications in Medicine and the Biological Sciences. New York, Dover Publications Inc., pp. 267-283, 1959.

18. Culver, W.E. Effects of cold on man. Physiol. Rev. 39:1-524, 1959.

19. Depocas, F. and R. Masironi. Body glucose as fuel for thermogenesis in the white rat exposed to cold. Amer. J. Physiol. 199:1051-1055, 1960.

20. Duffy, B.J. and H.R. Morgan. ACTH and cortisone aggravation or suppression of the febrile response of rabbits to bacterial endotoxin. Proc. Soc. Exp. Biol. and Med. 78:687, 1951.

21. Fregly, M.J. Interaction of adrenals and thyroid in maintenance of body temperature of rats exposed to cold. Amer. J. Physiol. 199:437-444, 1960.

22. Fuhrman, G.J. and F.A. Fuhrman. Effects of temperature on the action of drugs. Ann. Rev. Pharmacol. 1:65-78, 1961.

23. Garber, E. D. Nutritional aspects of infectious disease. Naval Research Reviews. Dept. of the Navy, Washington, D. C., 1960.

24. Girone, J. A. The effects of low temperature on bacterial infection. Mendel Bulletin 34:5-10. Villanova University Press, Villanova, Pa., 1962.

25. Halberg, F. and W. W. Spink. The influence of brucella somatic antigen (endotoxin) upon the temperature rhythm of intact mice. Lab. Investigation 5:283-294, 1956.

26. Hale, H. B. and R. B. Mefferd. Effects of somatotropin in rats acutely exposed to adverse environments. J. Appl. Physiol. 16:123-126, 1961.

27. Hardy, J. D. Physiology of temperature regulation. Physiol. Rev. 41:521-606, 1961.

28. Heroux, O. and J. S. Hart. Adrenal cortical hormone requirement of warm and cold acclimated rats after adrenalectomy. Amer. J. Physiol. 178:449-552, 1954a.

29. Heroux, O. and J. S. Hart. Cold acclimation and adrenal cortical activity as measured by eosinophil levels. Amer. J. Physiol. 178:453-456, 1954b.

30. Ingle, D. J. Endocrine mechanisms in adaptation to cold. Fed. Proc. 17:1064-1065, 1958.

31. Itoh, S., Y. Toyomasu and T. Konno. Water diuresis in cold environment. Japanese J. of Physiol. 9:438-443, 1959.

32. Kun, E. Effect of bacterial endotoxin on glycogen synthesis. Proc. Soc. Exp. Biol. and Med. 68:496-498, 1948.

33. Kun, E. and C. P. Miller. Effect of bacterial endotoxins on carbohydrate metabolism of rabbits. Proc. Soc. Exp. Biol. and Med. 67:221-225, 1948.

34. Long, C. N. H., B. Katzin and E. D. Fry. The adrenal cortex and carbohydrate metabolism. Endocrinology 26:309, 1940.

35. Miraglia, G. and L. J. Berry. Enhancement of Salmonellosis and emergence of secondary infection in mice exposed to cold. J. Bact. 84 (6):1173-1180,1962.

36. Miraglia, G. and L.J. Berry. Possible source of secondary invading staphylococci in mice exposed to acute cold. J. Bact. 85 (2): 345-348, 1963.

37. Previte, J.J. and L.J. Berry. The effect of environmental temperature on the host-parasite relationship in mice. J. Infect. Dis. 110: 201-209, 1962.

38. Rossiter, R.J. and D. Nicholls. Phosphorous metabolism of the adrenal gland of rats exposed to a cold environment. Revue Canadienne de Biologie 16:249-268, 1957.

39. Smith, I. and R. Dubos. The effect of nutritional disturbances on the susceptibility of mice to staphylococcal infections. J. Exp. Med. 103:109-118, 1956.

40. South, F.E. The effects of hot and cold environments on mammals. Proc. Animal Care Panel 10:51-56, 1960.

41. White, C. The use of ranks in a test for significance for comparing two treatments. Biometrics 8:33-41, 1952.

Arctic Aeromedical Laboratory, United States Air Force (AFSC), APO 731, Seattle, Wash.
Rpt. AAL-TDR-62-63. STUDIES ON THE POTENTIATION OF ENDOTOXIN IN MICE BY EXPOSURE TO COLD. May 1963, 17 p. incl. tables, 41 refs.
Unclassified Report

Mice exposed to 5° C show a drop in body temperature at a time when they are more sensitive than normal mice to bacterial lipopolysaccharide (LPS). Injection of 5 mg cortisone acetate protects against the lethal effects while 8 units but not 2 units of ACTH enhance lethality. These data suggest that adrenocortical exhaustion due to cold is in part responsible for the altered sensitivity to LPS. In support of this interpretation is the increase in urine volume and urinary nitrogen excreted by mice at 5° C compared with those at 25° C. Endotoxin decreases both. The decrease is counteracted by cortisone administration but not by ACTH

1. Toxins and Antitoxins
2. Mice
3. Exposure
4. Body Temperature
5. Stress (Physiology)
6. Corticosteroid Agents
7. Adrenocorticotropic Hormone
8. Urine
9. Nitrogen Compounds

I. Project 8241-01
II. Contract AF41(657)-340
III. Bryn Mawr College, Bryn Mawr, Pa.
IV. Previte, J. J. and L. J. Berry
V. Available from OTS
VI. In ASTIA collection

Arctic Aeromedical Laboratory, United States Air Force (AFSC), APO 731, Seattle, Wash.
Rpt. AAL-TDR-62-63. STUDIES ON THE POTENTIATION OF ENDOTOXIN IN MICE BY EXPOSURE TO COLD. May 1963, 17 p. incl. tables, 41 refs.
Unclassified Report

Mice exposed to 5° C show a drop in body temperature at a time when they are more sensitive than normal mice to bacterial lipopolysaccharide (LPS). Injection of 5 mg cortisone acetate protects against the lethal effects while 8 units but not 2 units of ACTH enhance lethality. These data suggest that adrenocortical exhaustion due to cold is in part responsible for the altered sensitivity to LPS. In support of this interpretation is the increase in urine volume and urinary nitrogen excreted by mice at 5° C compared with those at 25° C. Endotoxin decreases both. The decrease is counteracted by cortisone administration but not by ACTH

1. Toxins and Antitoxins
2. Mice
3. Exposure
4. Body Temperature
5. Stress (Physiology)
6. Corticosteroid Agents
7. Adrenocorticotropic Hormone
8. Urine
9. Nitrogen Compounds

I. Project 8241-01
II. Contract AF41(657)-340
III. Bryn Mawr College, Bryn Mawr, Pa.
IV. Previte, J. J. and L. J. Berry
V. Available from OTS
VI. In ASTIA collection